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NOTE ON THE STAGE OF *PIROPLASMA BIGEMINUM* WHICH OCCURS IN THE CATTLE TICK, *MARGAROPUS ANNULATUS*

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The organism herein described, which is believed to be a stage in the life cycle of *Piroplasma bigeminum,* was found in engorged female cattle ticks (*Margaropus annulatus*) removed from cattle in September, 1913. These ticks, which were being used as controls in a series of experiments at the Bureau of Animal Industry Experiment Station, Bethesda, Md., on the action of arsenical dips, had been immersed in water, dried, and maintained in the laboratory in petri dishes. Under such circumstances cattle ticks ordinarily behave in the normal manner; that is, by far the greater number live until oviposition is fully completed. In the present instance, however, certain of the lots showed an unusual mortality, as a result of which microscopical preparations were made and the organism which is the subject of the present paper discovered.

This, as seen in the figure, is a cigar-shaped body, with one end pointed and the other differentiated into a sort of cap. The ratio between the length and breadth varied considerably, but it is probable that this variation is due at least in part to the exigencies of fixation.

The cap, which is placed at what is probably the anterior end, varies a good deal in appearance in the different specimens. In some cases it has the shape of a crown with a minute, pointed process arising from its median portion (*A*). In others it consists merely of a narrow shell fitting over the broad end of the parasite (*D*), while again it consists of a rounded body resting upon the balance of the cell like the protomerite of a polycystid gregarine (*B, C*). As a matter of fact, these parasites are strikingly like minute gregarines, and may appropriately be termed gregarinoids. In some cases (*A, D*) the substance of the cap was much denser and more homogeneous than that of the balance of the cell, whereas in other cases (*B, C*) this distinction was not evident.

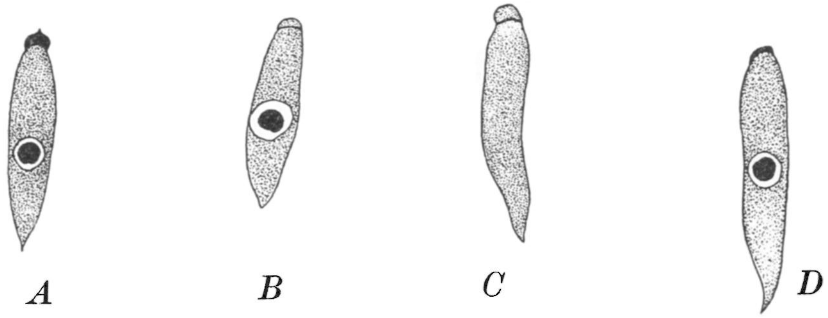
The cytoplasm is finely alveolar, thus following the protozoan type. In many of the specimens there were large vacuities, but these are probably to be accredited to the technique. An ectoplasmic layer is probably present, but it was not possible to demonstrate it satisfactorily.

The nucleus occupies a median position. It is of the vesicular type and inclosed within a thick and very distinct membrane. Within, a large rounded karyosome is always present.

Fifty specimens, selected at random, were measured. The extremes in length were 7μ and 15μ; the mean length 11.2μ. The distribution of these fifty specimens, with reference to their length, was as follows:

Length in microns	No. of Specimens
7	1
8	1
9	6
10	10
11	10
12	13
13	4
14	4
15	1

Christophers (1907) has described the life cycle of *Piroplasma canis* in the dog tick (*Rhipicephalus sanguineus*). In this, one of the most conspicuous phases is what Christophers calls the club-shaped body, which he describes on pages 56 to 61, and illustrates by a number of figures on his plate 2. The precise resemblance between these, and the forms herein described from *Margaropus annulatus,* leave no doubt as to their being corresponding stages in the life histories of *Piroplasma canis* and *Piroplasma bigeminum.*



According to Chistophers, the club-shaped bodies are derived directly from the parasites ingested by the tick in blood from the dog. The difference in both size and morphology between the intracellular form of *Piroplasma canis* and the club-shaped bodies is considerable, but Christophers figures several intermediate stages, and there seems to be no reason to question his conclusions. The club-shaped bodies were found in the gut of the dog tick, and, more abundantly, in the ovaries, oviducts, and eggs. Christophers describes two forms, as follows:

"(a) Rather rigid thorn-like bodies resembling on a very small scale certain gregarine trophozoites. This resemblance is largely due to the presence of a peculiar disc-like structure armed with cusps carried at the anterior extremity. They are sometimes motionless, but usually exhibit rather slow movements, especially a side to side flap-like action of the tail portion. The protoplasm is transparent, slightly refractile, and free from large granulations, and in it a clear area (the nucleus) can often be made out.

"The disc has the appearance of a boring organ. It carries four or five cusps, one of which is situated centrally, whilst the others are arranged around the periphery. Immediately behind this structure the parasite is often constricted so that a kind of neck is formed.

"(b) Leech-like forms more club-shaped than those just described and executing very active movements. They possess a swollen end which is often attached to the slide or cover glass and a thin end which is kept in constant motion like the head of a leech. They also undergo modified ameboid movements, the shapes depicted in plate 2, figure 2, being characteristic.

"These bodies are most numerous as a rule about the oviducts, and a few can be found here when not to be detected elsewhere. They also occur in the ovary and may be seen lying with ova not yet free in the lumen, and in more mature ova. By careful search they can also be found in fresh preparations of the gut.

"In stained preparations a greater variety of forms is apparent, but the two forms mentioned, those with and those without a disc, are distinguishable. Since intermediate stages are not difficult to find it is probable that both are identical in nature, the disc bearing forms being the more mature stage."

Finally, according to Christophers, these club-shaped bodies enlarge, become irregularly rounded, and transform themselves into what he designates as the zygote. This term, however, is singularly unfortunate, since no sexual process is described. The "zygotes" by a series of steps which Christophers describes in detail, break up into sporozoites, these later stages of the life cycle taking place in the salivary glands of the tick.

Koch (1906), studying *P. bigeminum,* figured and described what are clearly the same bodies, although he says nothing about the curious cap or crown present at one end of the parasite. Koch found these elements both in engorged female ticks three days after removal from the cow, and in the eggs. According to his account, they are the later rather than the earlier stages in the evolution of *Piroplasma* in the cattle tick, but he did not follow them further than the stage here under consideration.

Kleine (1906) and Nuttall and Graham-Smith (1908) cultured *Piroplasma canis,* and obtained developmental forms much like the earlier stages of *P. bigeminum* in the tick, as described by Koch. The cigar-shaped body, however, has so far only been seen in either ticks or tick eggs.

These various observations are not wholly accordant, nor do they seem to cover the entire process. Minchin (1912: 384), however, has endeavored to put them together and to formulate an outline of the life history of *Piroplasma* in the tick, and to this the reader is referred.

My own observations on *Piroplasma bigeminum* in *Margaropus annulatus* concern only the club-shaped bodies or gregarinoids. As already stated, these differ somewhat amongst themselves with regard to the morphology of the cap at the anterior end, but, as Christophers suggests, such differences are probably merely a matter of the stage of development. In my material from *Margaropus annulatus,* the condition of the cap shown in *D,* was quite infrequent, from which it may be surmised that these gregarinoids were in an earlier rather than a later stage of their evolution. Furthermore, as already stated, the gregarinoid was the only stage that I was able to find, or, at least, to recognize.

In addition to their occurrence in smears made from the ticks themselves, the gregarinoids were also found in preparations made from the crushed eggs. In the eggs, however, they were very scarce and could be picked up only with the greatest difficulty. In crushed seed ticks, hatched from eggs laid by ticks of the infected lots, I have not been able to find either the gregarinoids or any element which can be identified as belonging to *Piroplasma bigeminum.*

It has been noted that the ticks in which the parasites were found had shown an unusually high mortality, it being on this account that they were examined. This suggests that the *Piroplasma* is pathogenic for the tick as well as for the cow.

In consequence of the discovery of the parasite of Texas fever in the tick, a series of experiments was carried out and, although they were wholly negative, the failure is of itself of some significance, and a brief résumé may not be out of place.

The ticks which showed the high mortality were collected early in September from a certain cow (No. 1040 of the Bureau of Animal Industry series). The microscopical preparations were made on September 22 from ticks collected on September 4, or 18 days after reaching maturity. The weather being then warm all of the ticks which had survived were either in the midst of oviposition or had nearly or quite completed it. It was obviously beside the point to search for the parasite in the bodies of ticks which had laid all their eggs since such ticks merely shrivel and die. Moreover, the material was not abundant.

In consequence, the eggs of the batches of ticks (collected September 2, 3, 4 and 5) which showed the high mortality, and which were moreover known to be infected, were kept in appropriate containers and permitted to hatch. On Oct. 18, 1913, microscopical preparations were made of a number of the seed ticks, and the balance of them were placed on cow 1040. These ticks began to come to maturity on November 24, and collections were made daily from November 24 to November 28. Microscopical preparations were made daily of these ticks from one to seventeen days after their removal from the cow, but the parasite could not be found. In consequence, the experiment was abandoned.

These negative results are in line with the results of observations made in 1908, during the course of which a number of infectious ticks were examined without detecting anything which could be identified as belonging to the life cycle of *Piroplasma bigeminum*. The inference seems to be that of a given number of so-called infectious cattle ticks, only a certain proportion actually harbor the parasite. It is easy to understand how this might take place. A female cattle tick lays several thousand eggs and in order that her entire brood should be infected it would be necessary that each egg receive at least one parasite. Such a condition could fail of realization in two ways. In the first place, the engorged female might not harbor as many parasites as eggs, and in the second, the distribution of the parasites would need to be remarkably accurate to insure that each and every egg became infected.

It is also evident, from the experiments herein outlined, that whereas a given lot of ticks may be heavily infected, the offspring of these may be negative at least so far as a microscopical examination is concerned.

It may finally be noted that a spirochaete has several times been detected in cattle ticks and that it was present in the lot which showed the heavy infection with *Piroplasma*. *Spirochaeta theileri,* first discovered by Theiler (1904) in cattle in Africa, is stated to be the causal agent of a mild disease. It is known to be transmissible by *Margaropus decoloratus,* and further that adult infected ticks of this species can transmit the spirochaete to their offspring. *Margaropus decoloratus* and *Margaropus annulatus,* the former in Africa and the latter in the United States, play similar rôles in the transmission of cattle diseases. Hence it may be suggested that the spirochaete occurring in *Margaropus annulatus* is *Spirochaeta theileri.*

SUMMARY

A parasitic protozoan was found in smears made from female cattle ticks (*Margaropus annulatus*), and from crushed eggs which they had deposited. The parasite has the form of a minute polycystid gregarine, and is believed to represent the stage of *Piroplasma bigeminum* occurring in the tick. It is essentially like the form figured and described by Koch as present in engorged female ticks and their eggs, and also like the form of *Piroplasma canis* found by Christophers in

Rhipicephalus sanguineus. In the present case, it is of interest to note that the female ticks in which the parasites were found showed an unusual mortality, suggesting that the parasite is pathogenic for the tick as well as for the cow. In addition to the gregarinoid parasite a spirochaete was found in the ticks. This parasite not heretofore reported from the United States is perhaps the same as the form known as *Spirochaeta theileri.*

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